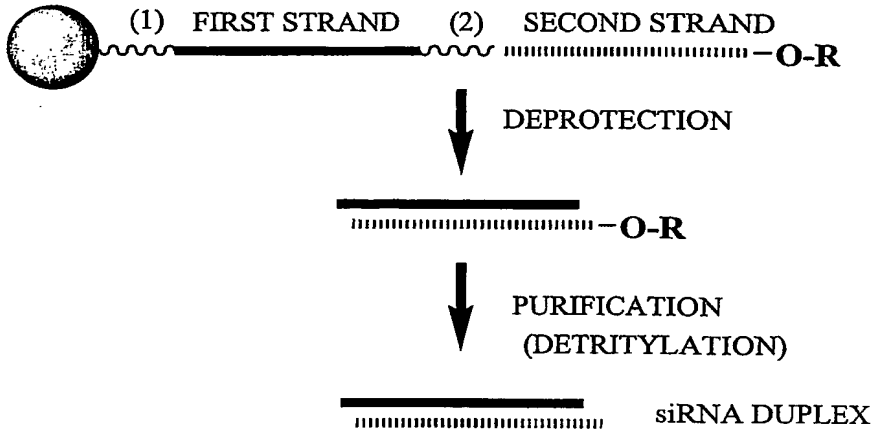
Figure 1

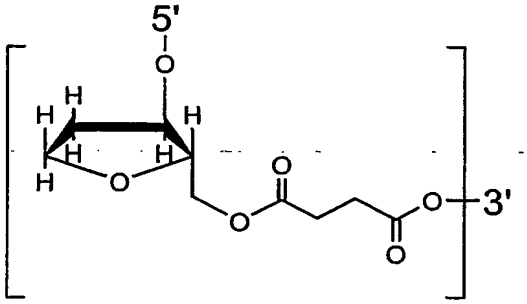


= SOLID SUPPORT

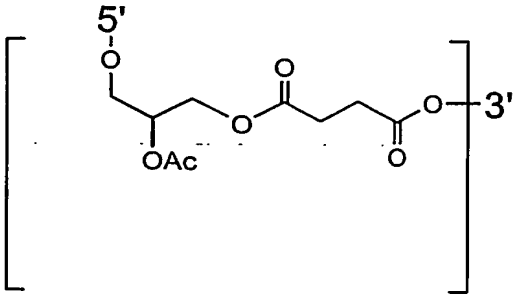
R = TERMINAL PROTECTING GROUP
FOR EXAMPLE:
DIMETHOXYTRITYL (DMT)

(1) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)

(2) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)



INVERTED DEOXYABASIC SUCCINATE LINKAGE



GLYCERYL SUCCINATE LINKAGE

Figure 2

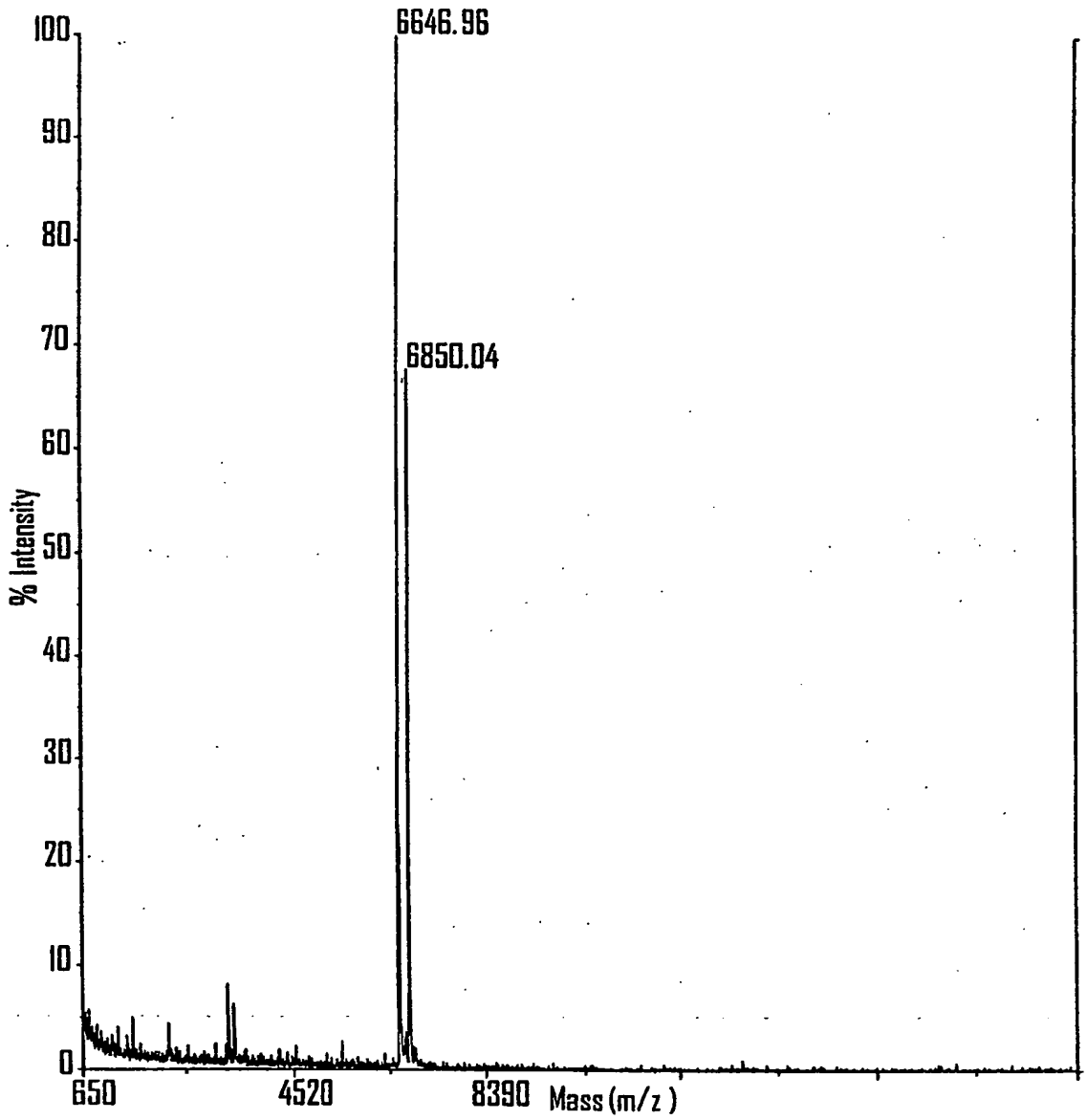

6646.96
6850.04
100
90
80
70
60
50
40
30
20
10
0
% Intensity
650
4520
8390
Mass (m/z)

Figure 3

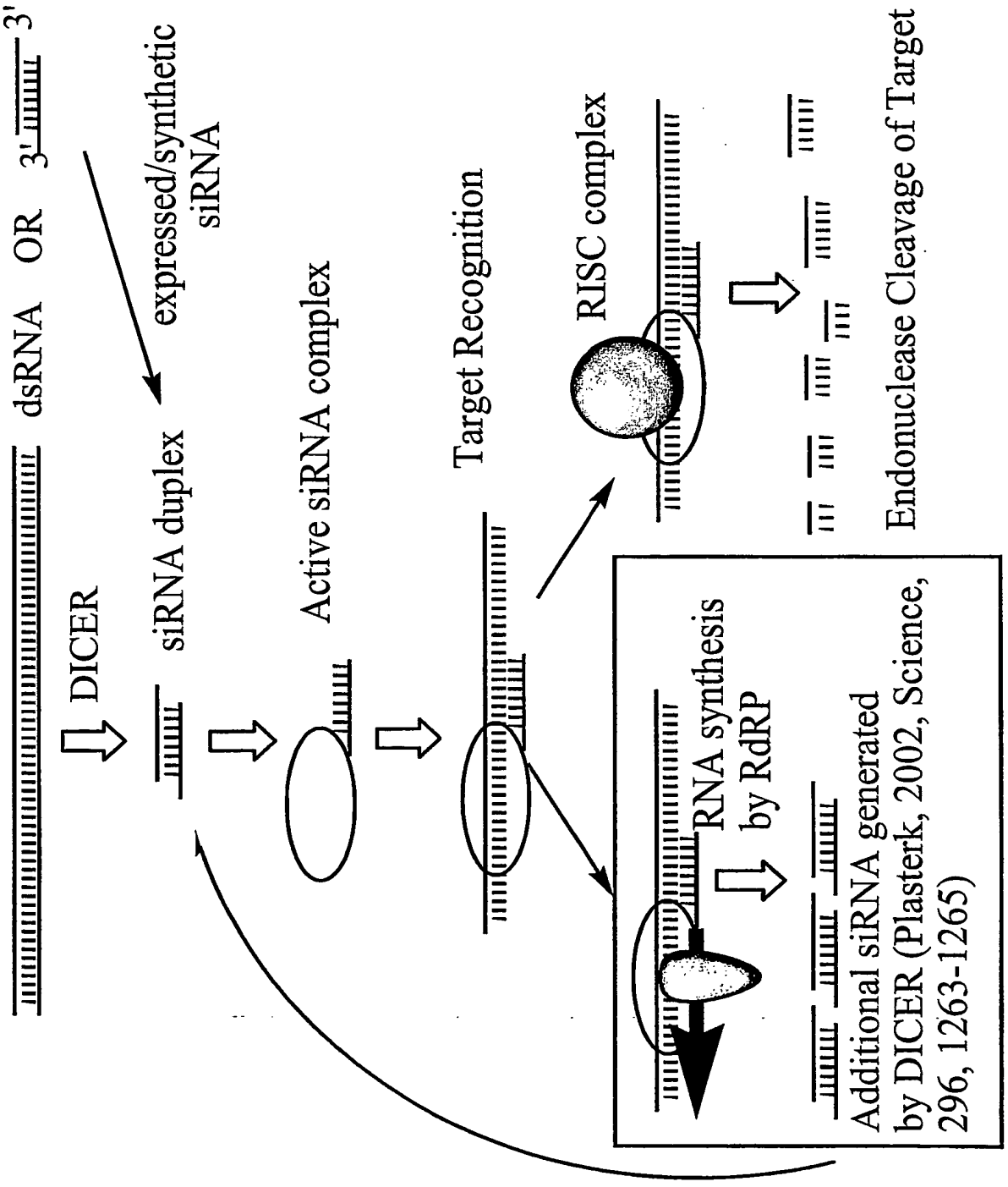

dsRNA OR 3' 3'
DICER
siRNA duplex
expressed/synthetic siRNA
Active siRNA complex
Target Recognition
RISC complex
RNA synthesis by RdRP
Endonuclease Cleavage of Target
Additional siRNA generated by DICER (Plasterk, 2002, Science, 296, 1263-1265)

Figure 4

A

SENSE STRAND (SEQ ID NO 305)
ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 306)
ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)

B

SENSE STRAND (SEQ ID NO 307)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-OME EXCEPT POSITIONS (N N)

5'- N N N N N N N N N N N N N N N N N N N (N_SN) -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 308)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

C

SENSE STRAND (SEQ ID NO 309)
ALL PYRIMIDINES = 2'-O-ME OR 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 310)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

D

SENSE STRAND (SEQ ID NO 311)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 308)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

E

SENSE STRAND (SEQ ID NO 312)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 308)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

F

SENSE STRAND (SEQ ID NO 311)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 313)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

POSITIONS (NN) CAN COMPRISE ANY NUCLEOTIDE, SUCH AS DEOXYNUCLEOTIDES (eg. THYMIDINE) OR UNIVERSAL BASES
B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT
L = GLYCERYL or B THAT IS OPTIONALLY PRESENT
S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE THAT IS OPTIONALLY PRESENT

Figure 5

A

SENSE STRAND (SEQ ID NO 314)

5'- B- C A G U G A U G G U G A A A U U C C U *T T*-B -3'

3'- L-T_S*T* G U C A C U A C C A C U U U A A G G A -5'

ANTISENSE STRAND (SEQ ID NO 315)

B

SENSE STRAND (SEQ ID NO 316)

5'- *c a g u g a u g g u g a a a u u c c u* T_S*T* -3'

3'- L-T_S*T g u c a c u a c c a c u u u a a g g a* -5'

ANTISENSE STRAND (SEQ ID NO 317)

C

SENSE STRAND (SEQ ID NO 318)

5'- B- c A G u G A u G G u G A A A u u c cu*TT*-B -3'

3'- L-T_ST G *u c A c u A c c A c u u u* A A G G A -5'

ANTISENSE STRAND (SEQ ID NO 319)

D

SENSE STRAND (SEQ ID NO 320)

5'- B- *c A G u G A u G G u G A A A u u c c u TT*-B -3'

3'- L-T_S*T g u c a c u a c c a c u u u a a g g a* -5'

ANTISENSE STRAND (SEQ ID NO 317)

E

SENSE STRAND (SEQ ID NO 321)

5'- B-*c* A G *u* G A *u* G G *u* G A A A *u u c c u TT*-B -3'

3'- L-T_S*T g u c a c u a c c a c u u u a a g g a* -5'

ANTISENSE STRAND (SEQ ID NO 317)

F

SENSE STRAND (SEQ ID NO 320)

5'- B- *c A G u G A u G G u G A A A u u c c u TT*-B -3'

3'- L-T_S*T G u c A c u A c c A c u u u A A G G A* -5'

ANTISENSE STRAND (SEQ ID NO 322)

lower case = 2'-O-Methyl or 2'-deoxy-2'-fluoro

italic lower case = 2'-deoxy-2'-fluoro

underline = 2'-O-methyl

ITALIC UPPER CASE = DEOXY

L = GLYCERYL MOIETY OR B OPTIONALLY PRESENT

B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT

S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE OPTIONALLY PRESENT

Figure 6

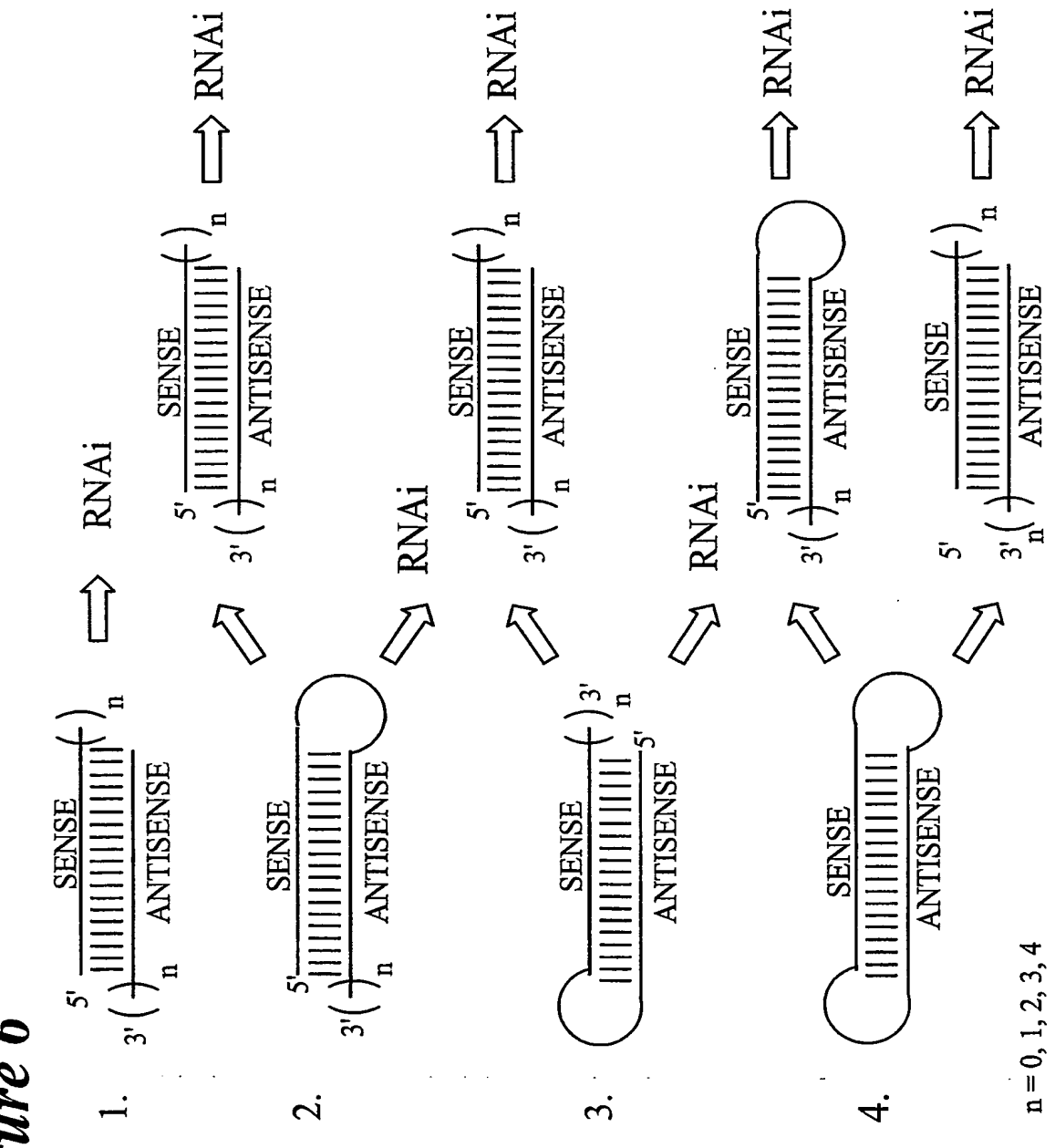


Figure 7

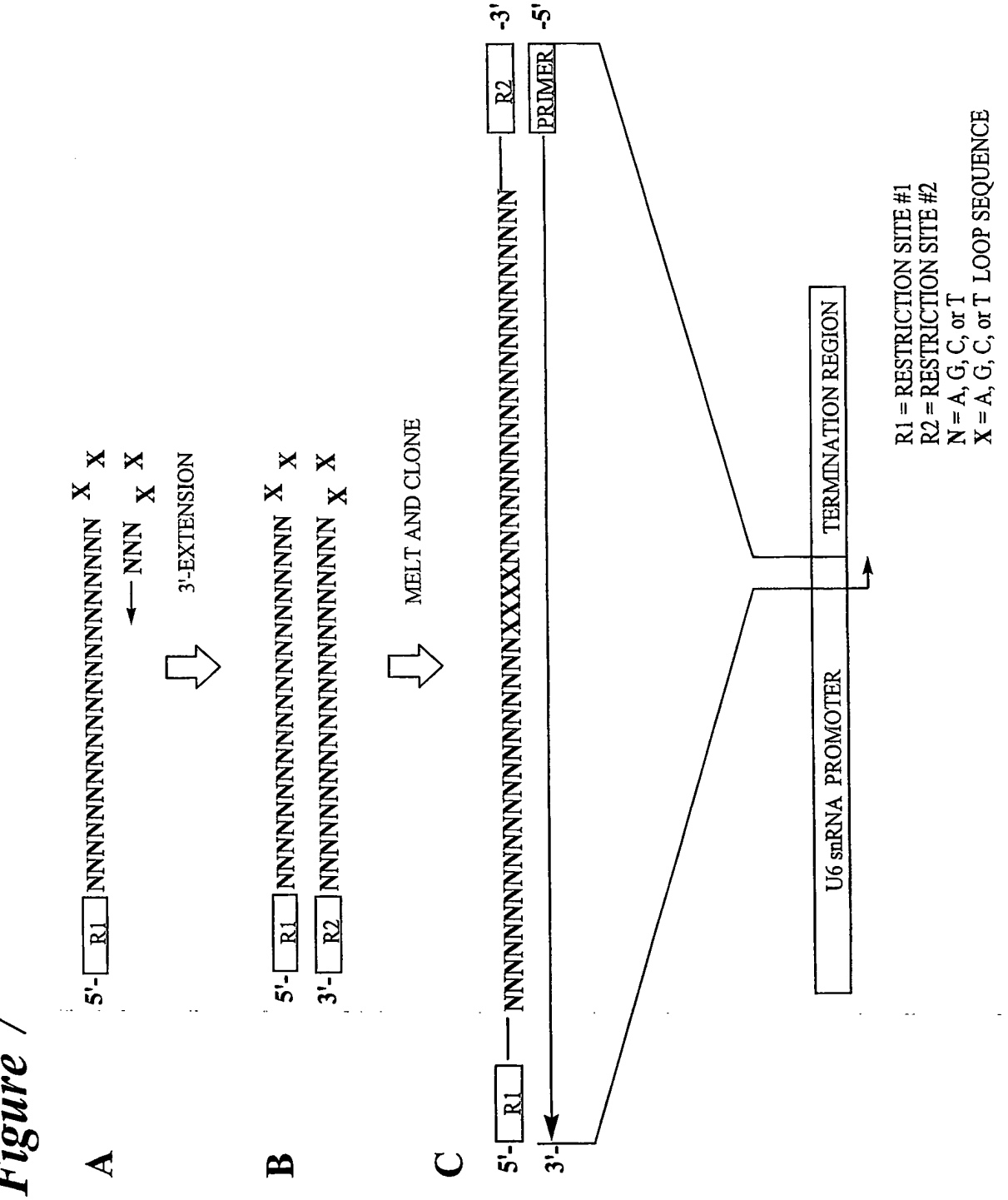
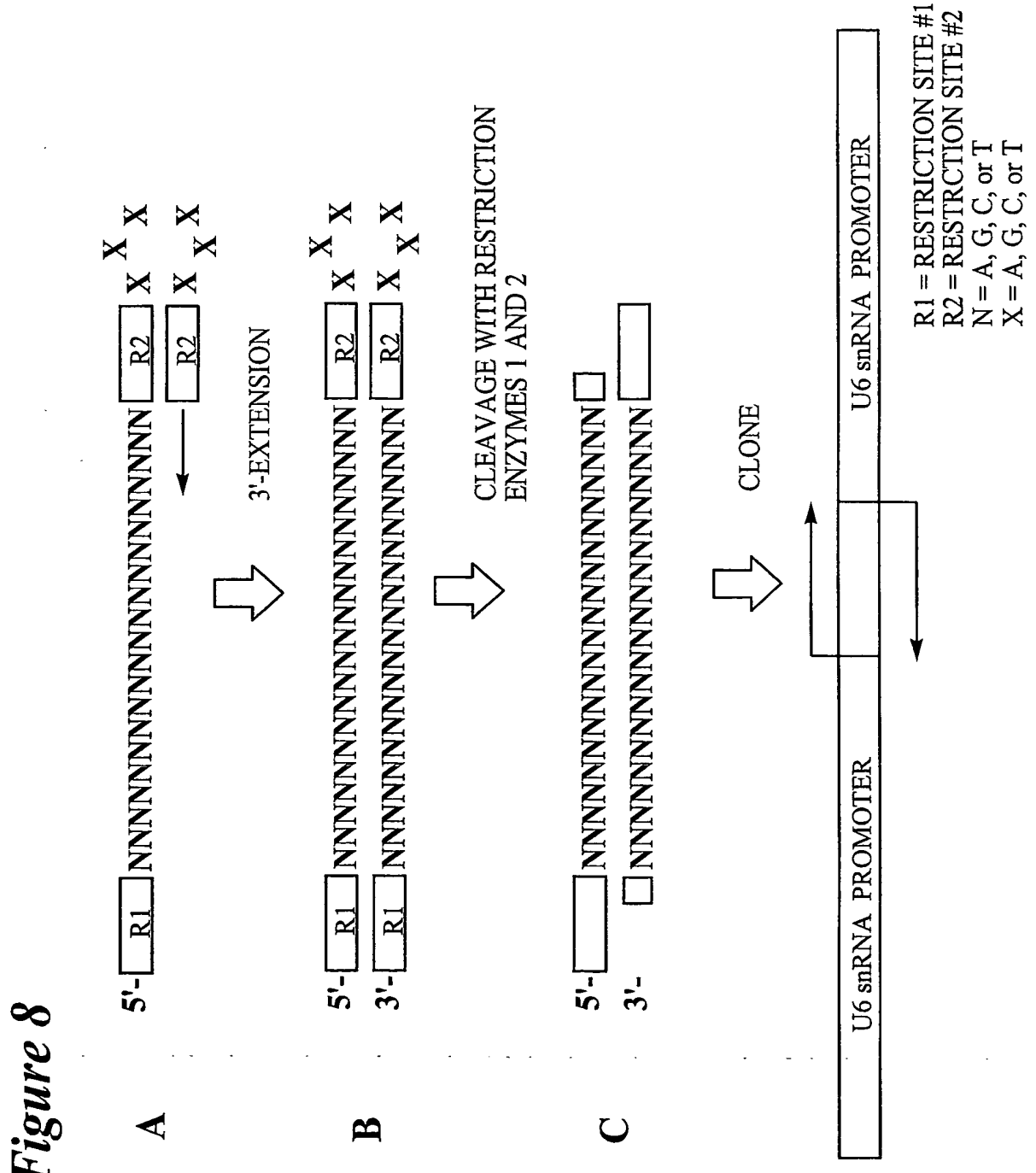

A
5'- R1 NNNNNNNNNNNNNNNNNNN X X
NNN X X
3'-EXTENSION
B
5'- R1 NNNNNNNNNNNNNNNNNNN X X
3'- R2 NNNNNNNNNNNNNNNNNNN X X
MELT AND CLONE
C
5'- R1 — NNNNNNNNNNNNNNNNNNNNXXXXNNNNNNNNNNNNNNNNNNN — R2 -3'
3'- PRIMER -5'
U6 snRNA PROMOTER
TERMINATION REGION
R1 = RESTRICTION SITE #1
R2 = RESTRICTION SITE #2
N = A, G, C, or T
X = A, G, C, or T LOOP SEQUENCE

Figure 8


A
5'- R1 NNNNNNNNNNNNNNNNNNN R2 X X
R2 X X
X X
3'-EXTENSION
B
5'- R1 NNNNNNNNNNNNNNNNNNN R2 X X
3'- R1 NNNNNNNNNNNNNNNNNNN R2 X X
CLEAVAGE WITH RESTRICTION ENZYMES 1 AND 2
C
5'- NNNNNNNNNNNNNNNNNNN
3'- NNNNNNNNNNNNNNNNNNN
CLONE
U6 snRNA PROMOTER
U6 snRNA PROMOTER
R1 = RESTRICTION SITE #1
R2 = RESTRICTION SITE #2
N = A, G, C, or T
X = A, G, C, or T

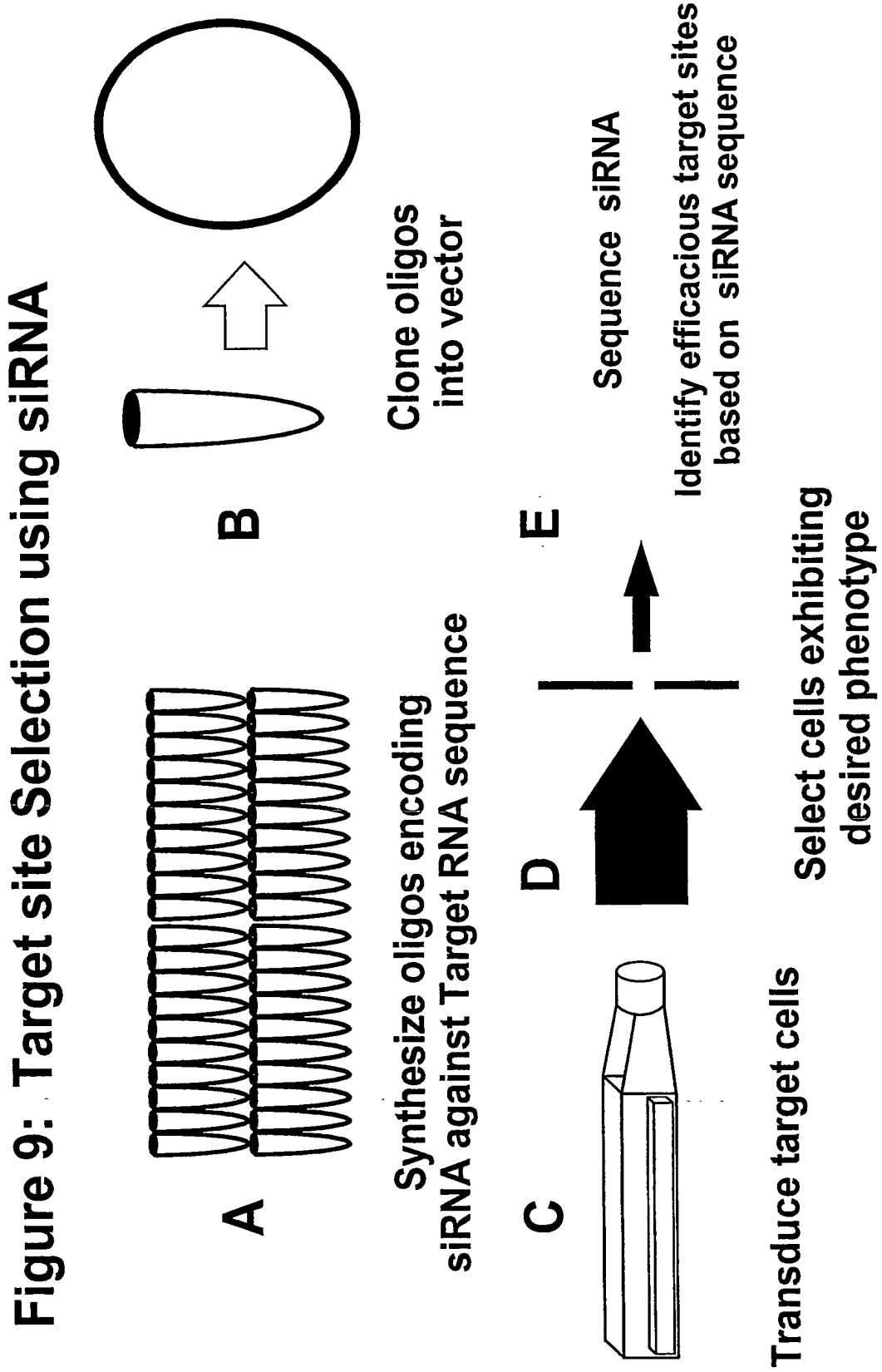
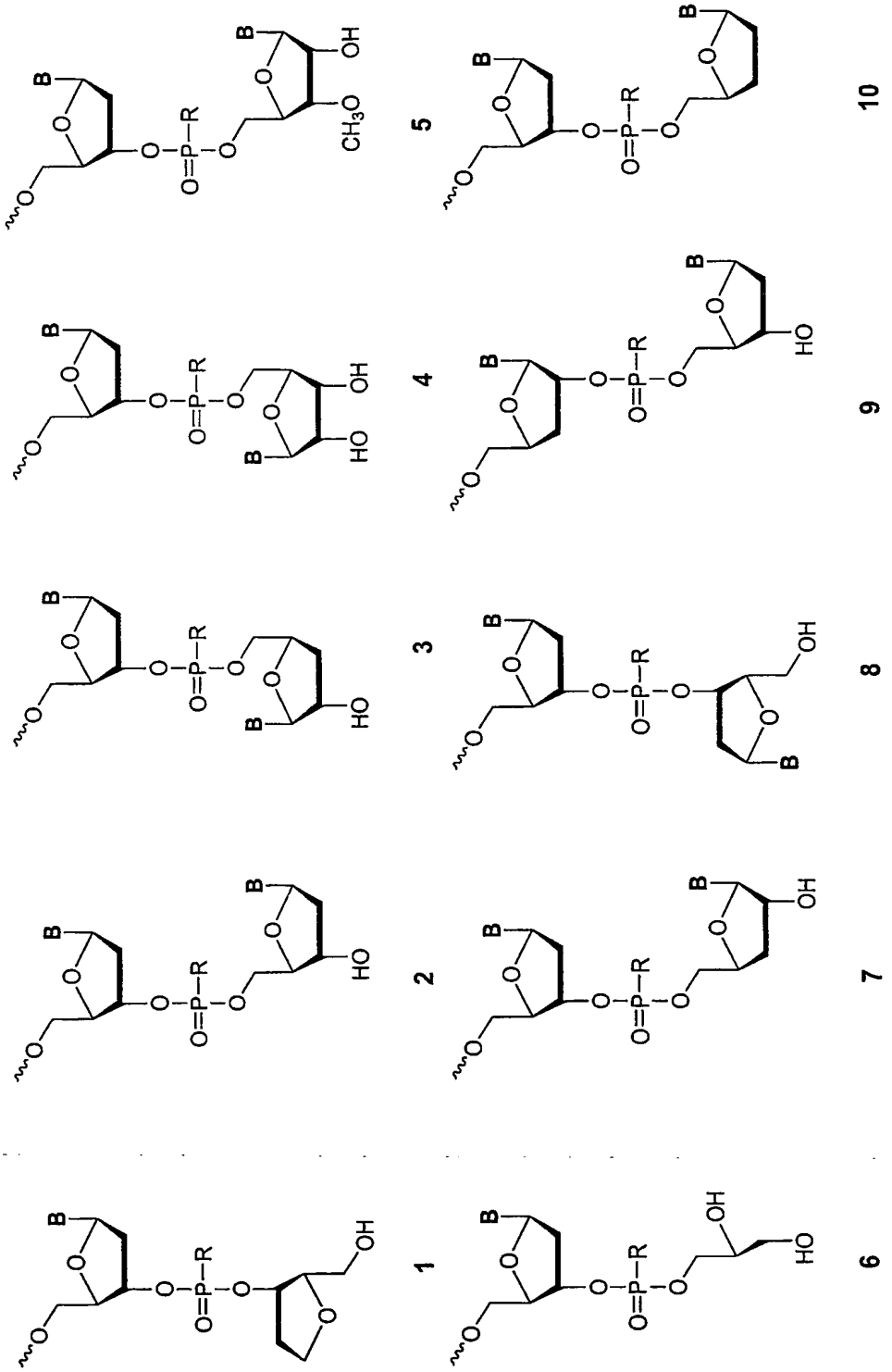


Figure 9: Target site Selection using siRNA
A
Synthesize oligos encoding siRNA against Target RNA sequence
B
Clone oligos into vector
C
Transduce target cells
D
Select cells exhibiting desired phenotype
E
Identify efficacious target sites based on siRNA sequence
Sequence siRNA

Figure 10



R = O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl
B = Independently any nucleotide base, either naturally occurring or chemically modified, or optionally H (abasic).

Figure 11: Modification Strategy

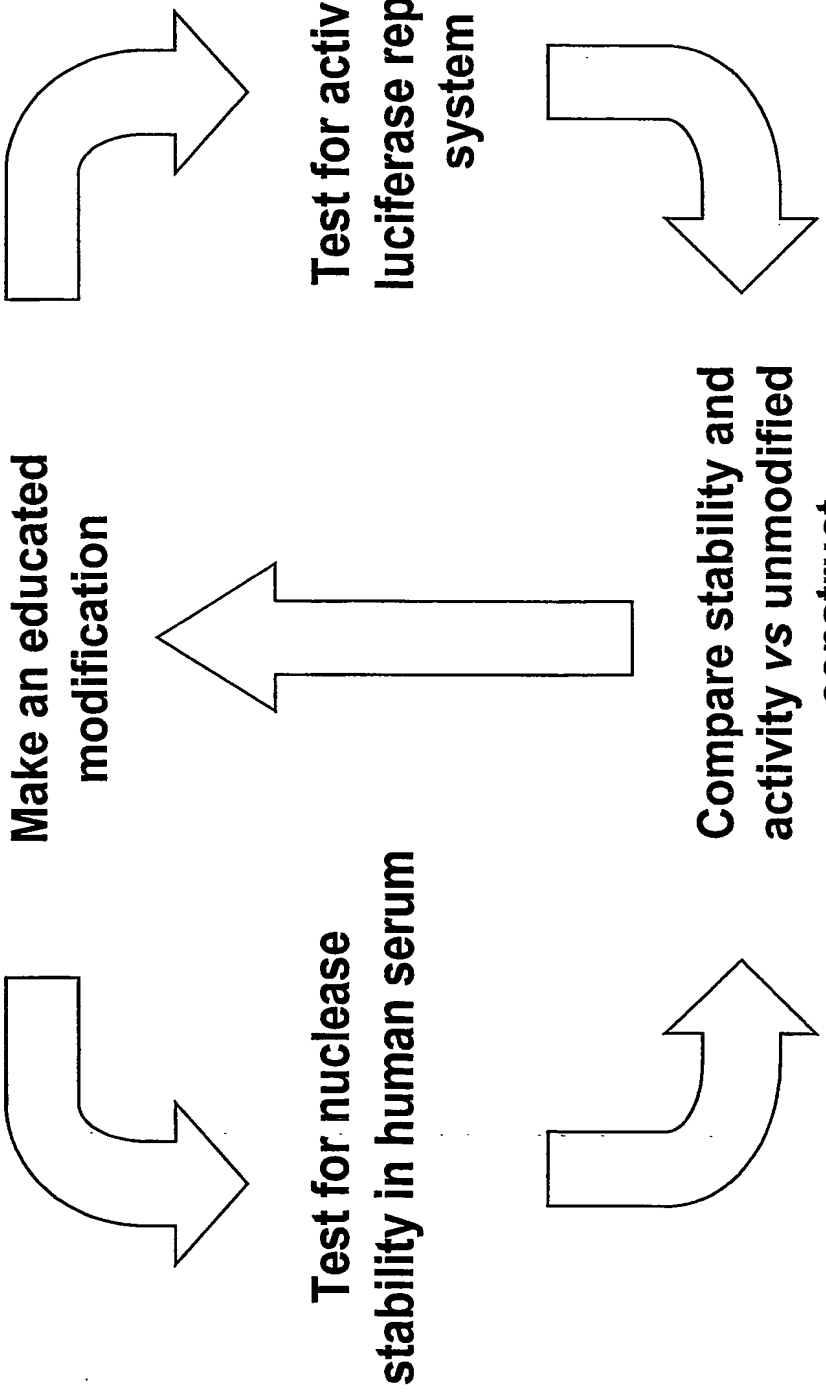
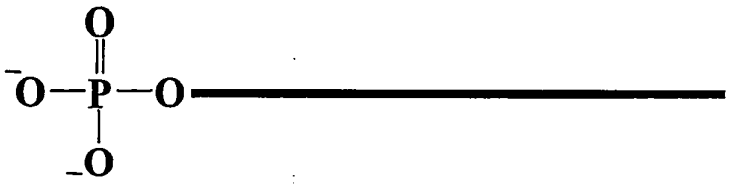
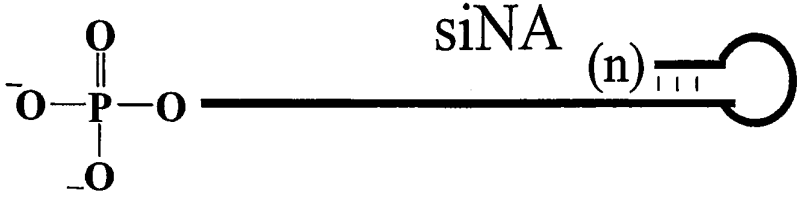


Figure 12: Phosphorylated siNA constructs

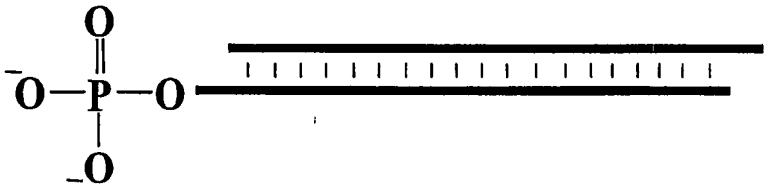


Phosphates can be modified as described herein

Asymmetric hairpin siNA



Asymmetric duplex siNA



(n) = number of base pairs (e.g. 3-18 bp)

Figure 13: 5'-phosphate modifications

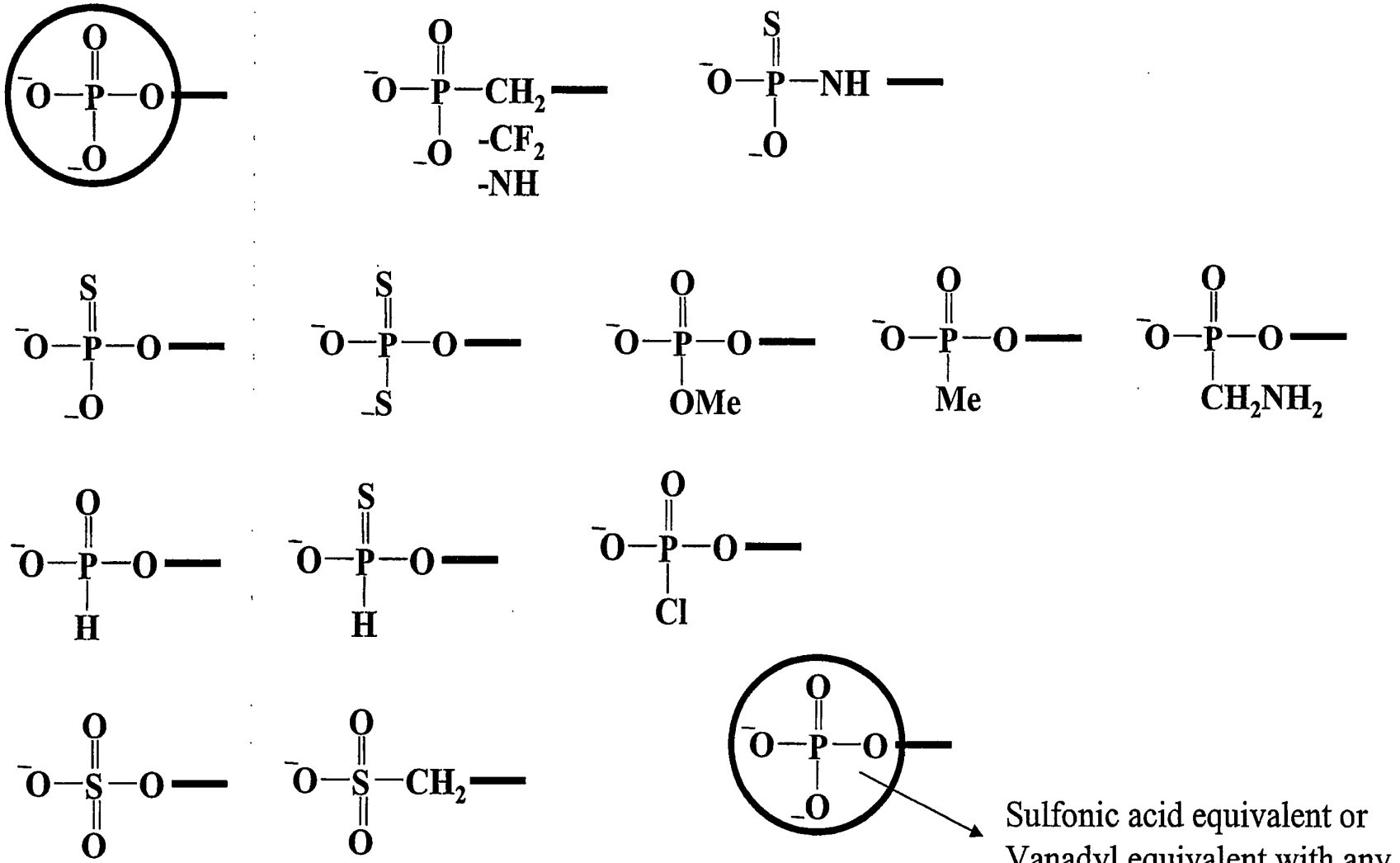


Figure 14A: Duplex forming oligonucleotide constructs that utilize Palindrome or repeat sequences

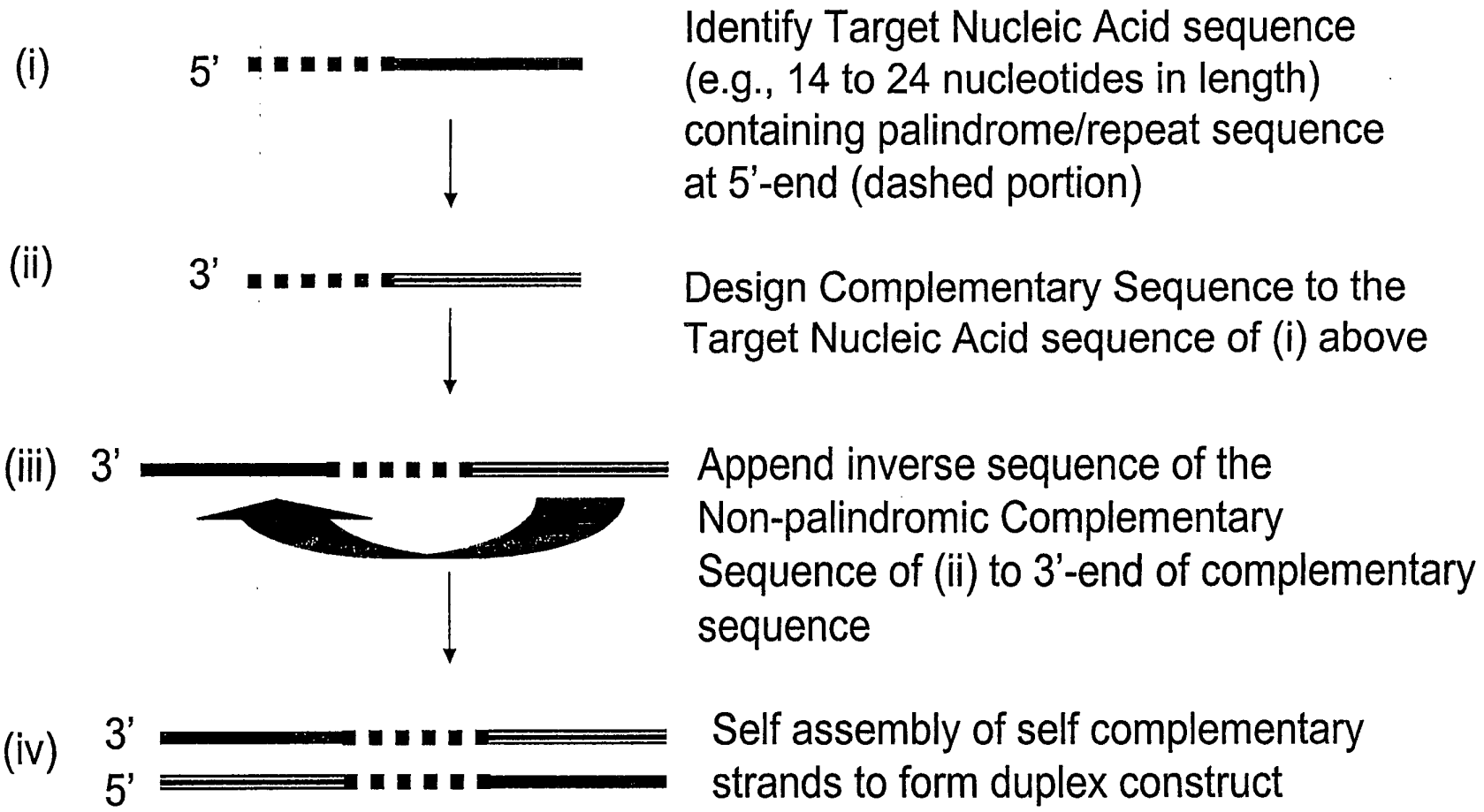
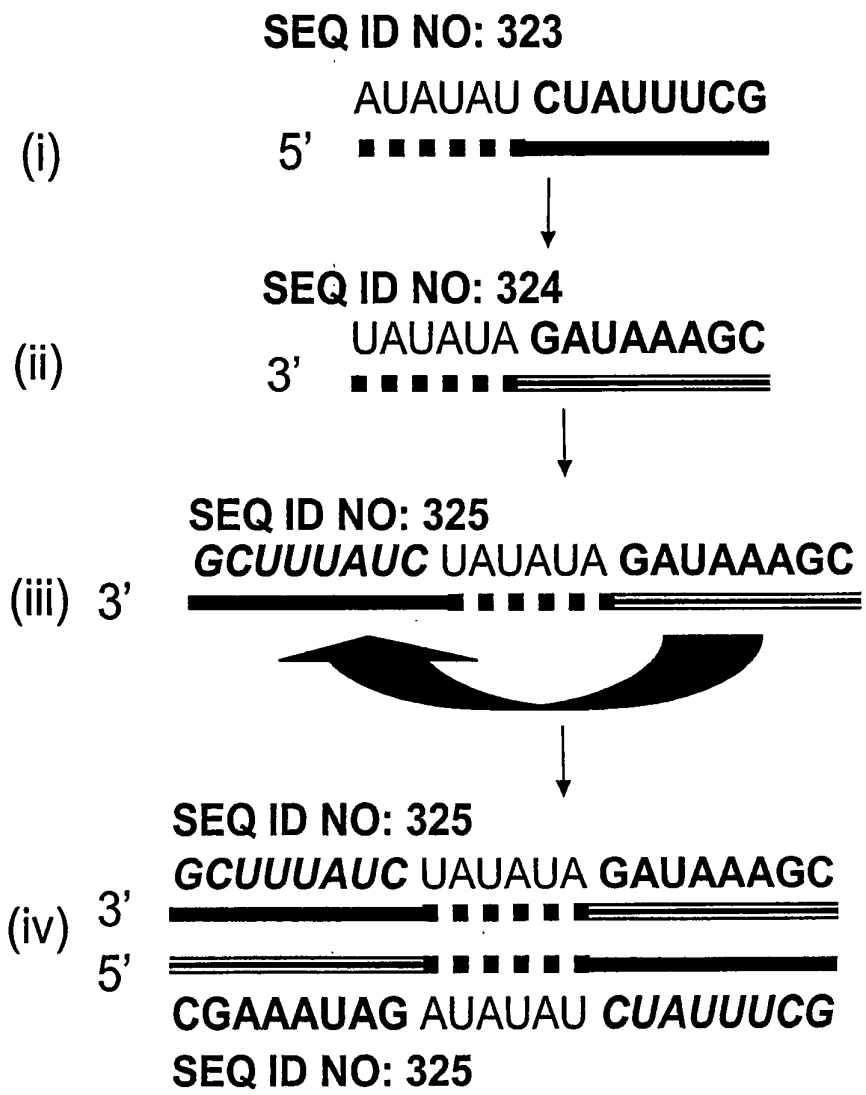


Figure 14B: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence



Identify Target Nucleic Acid sequence (e.g., 14 to 24 nucleotides in length) containing palindrome/repeat sequence at 5'-end (dashed portion)

Design Complementary Sequence to the Target Nucleic Acid sequence of (i) above

Append inverse sequence of the Non-palindromic Complementary Sequence of (ii) to 3'-end of complementary sequence

Self assembly of self complementary strands to form duplex construct (blunt ends)

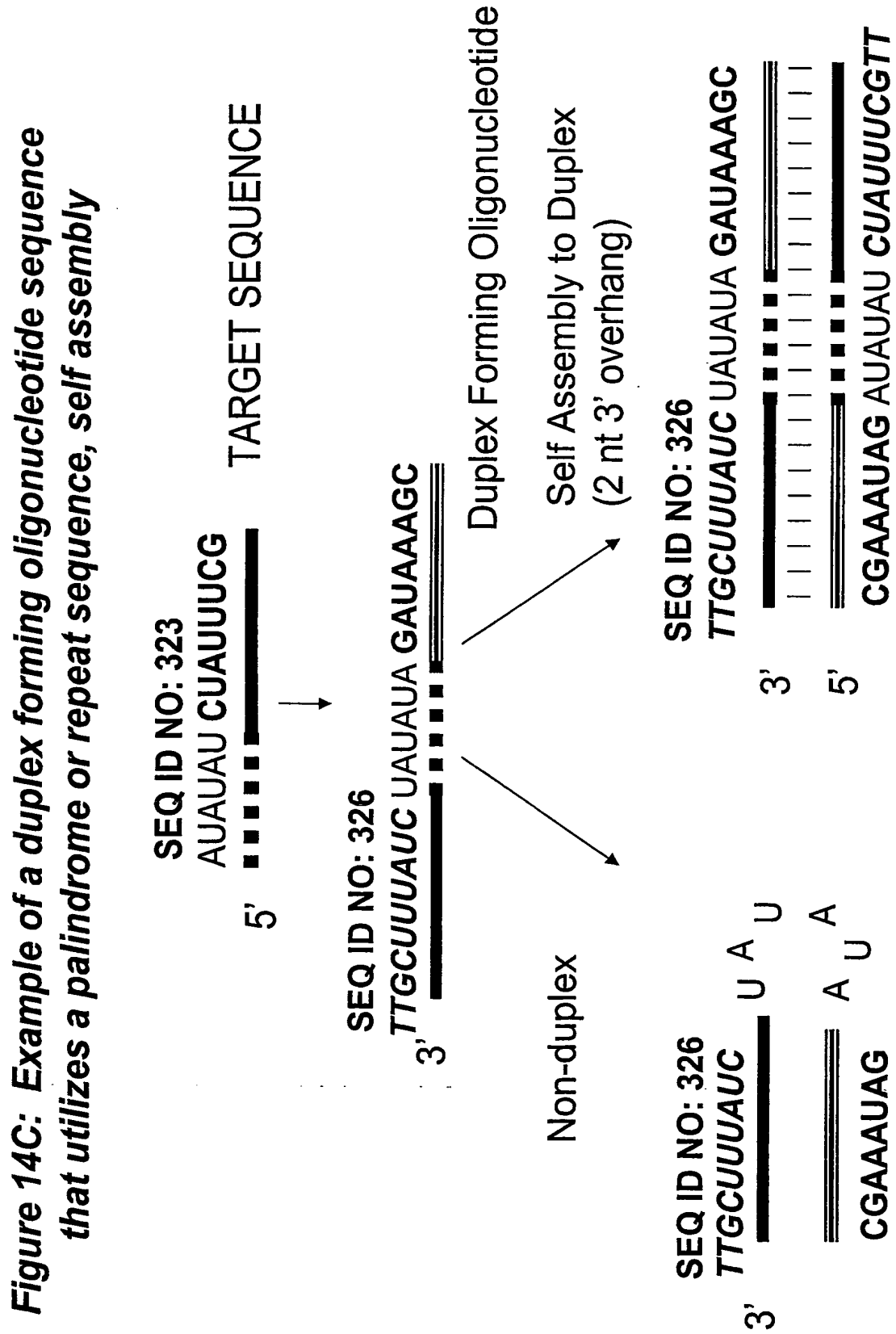


Figure 14C: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly

Figure 14D: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly and inhibition of Target Sequence Expression

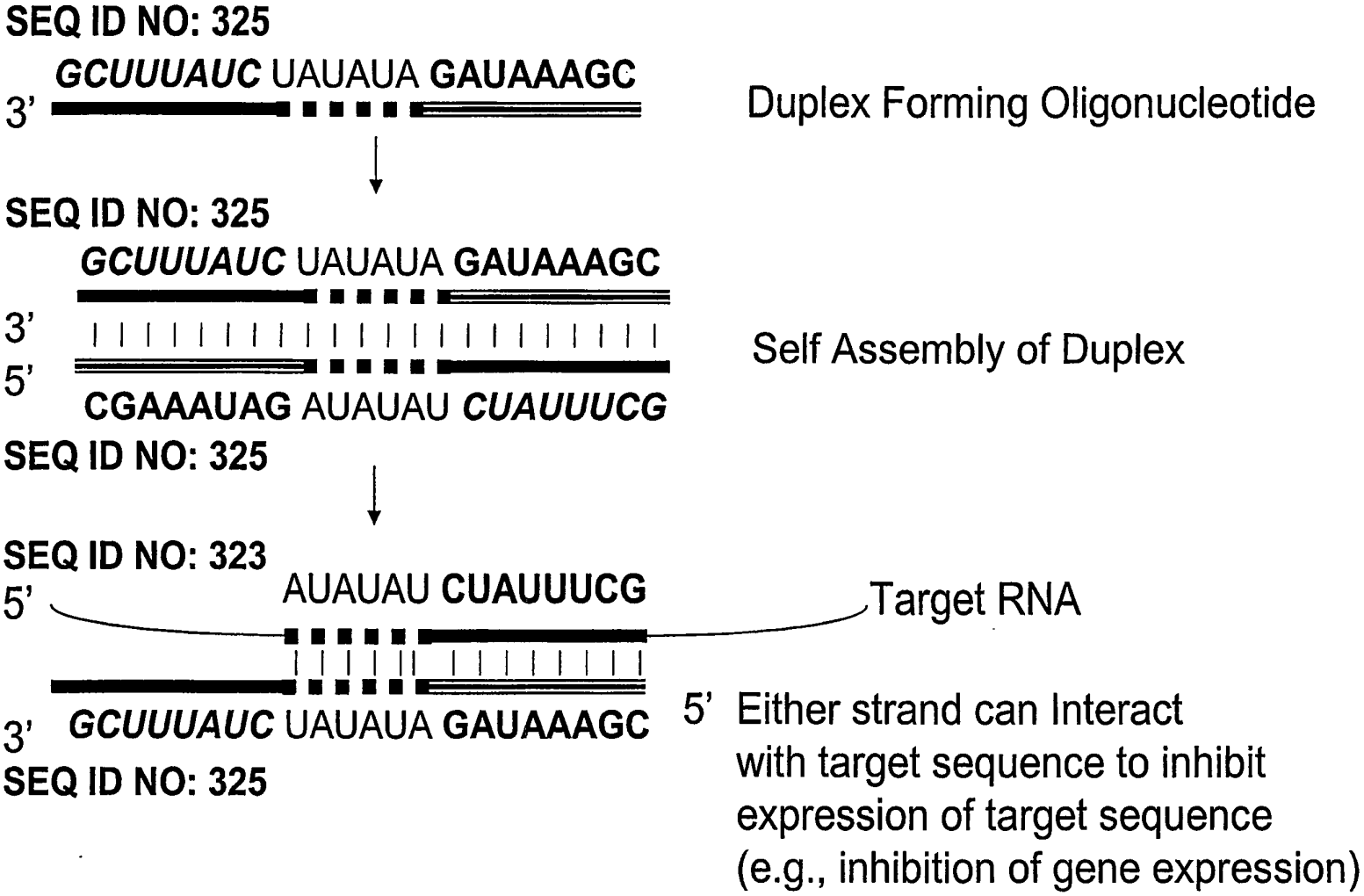
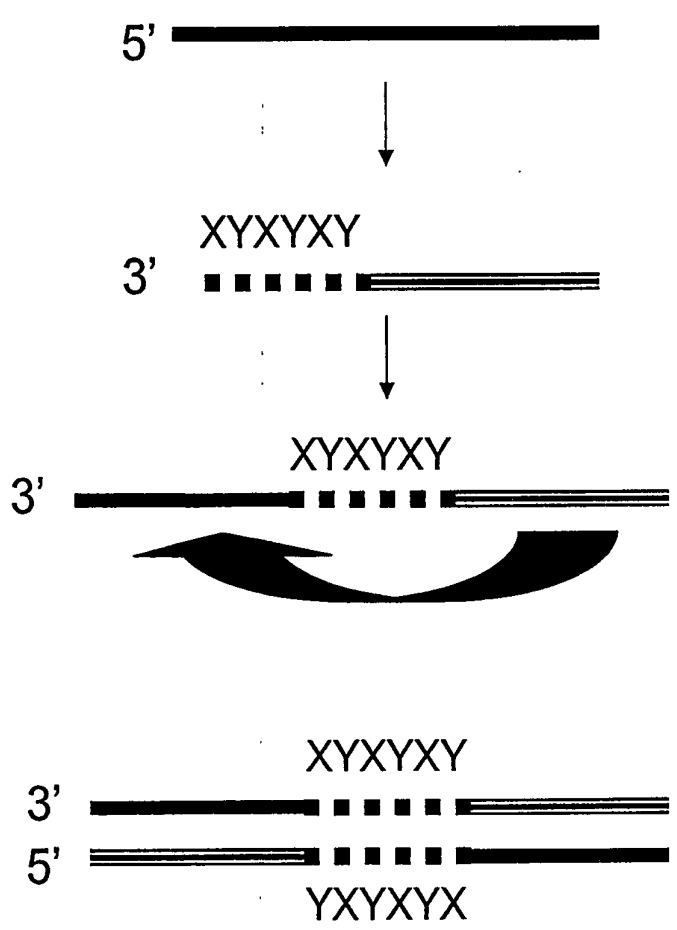


Figure 15: Duplex forming oligonucleotide constructs that utilize artificial palindrome or repeat sequences



Identify Target Nucleic Acid sequence (e.g., 14 to 24 nucleotides in length)

Design Complementary Sequence and utilize modified nucleotides (shown as X, Y) that interact with a portion of the target sequence and result in the formation of a palindrome/repeat sequence (e.g., 2 to 12 nucleotides) at 3'-end (dashed portion)

Append inverse sequence of Complementary region to 3'-end of palindrome/repeat sequence

Hybridize self complementary strands to form duplex siNA construct

Figure 16: Examples of double stranded multifunctional siNA constructs with distinct complementary regions

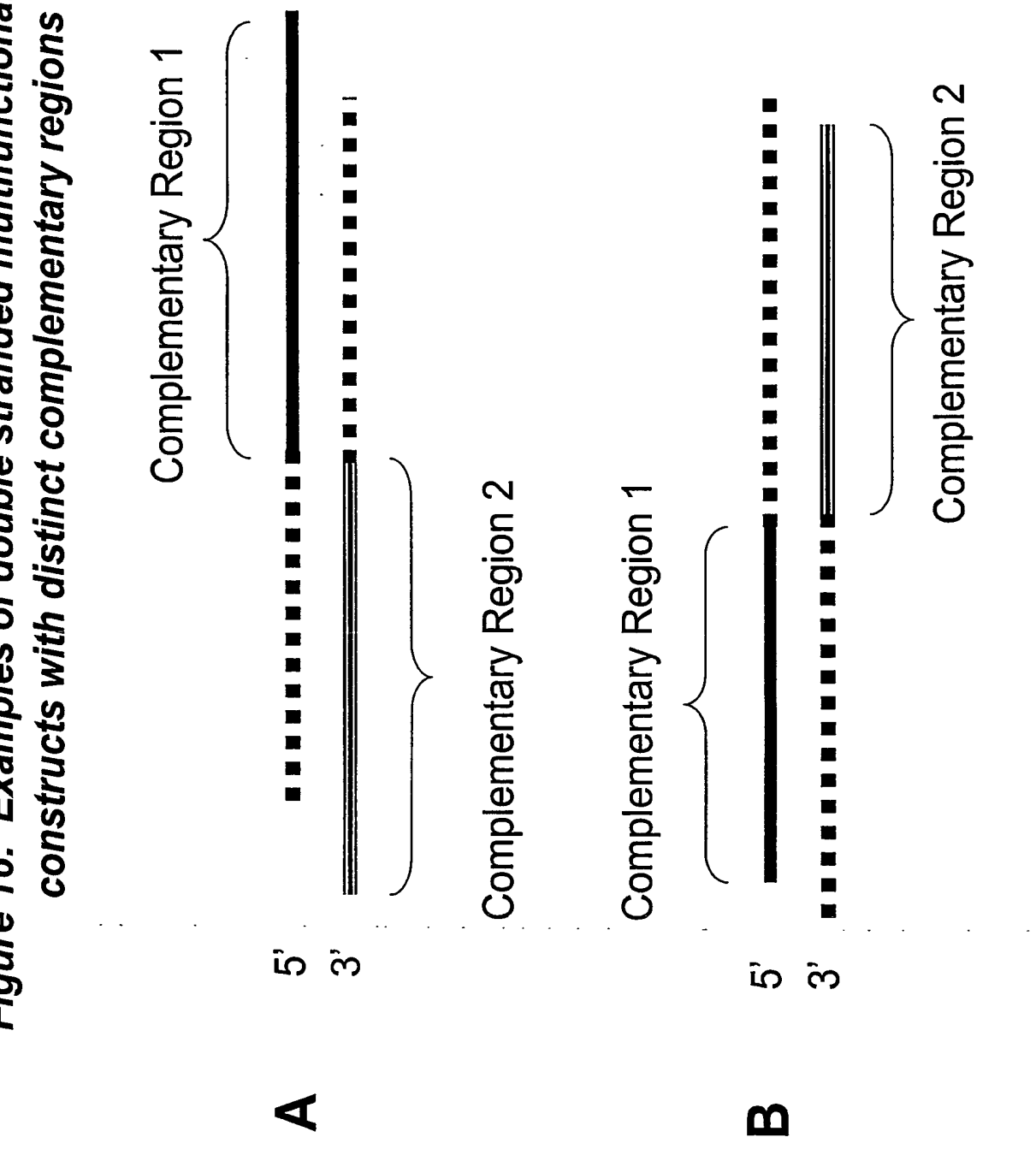


Figure 17: Examples of hairpin multifunctional siNA constructs with distinct complementary regions

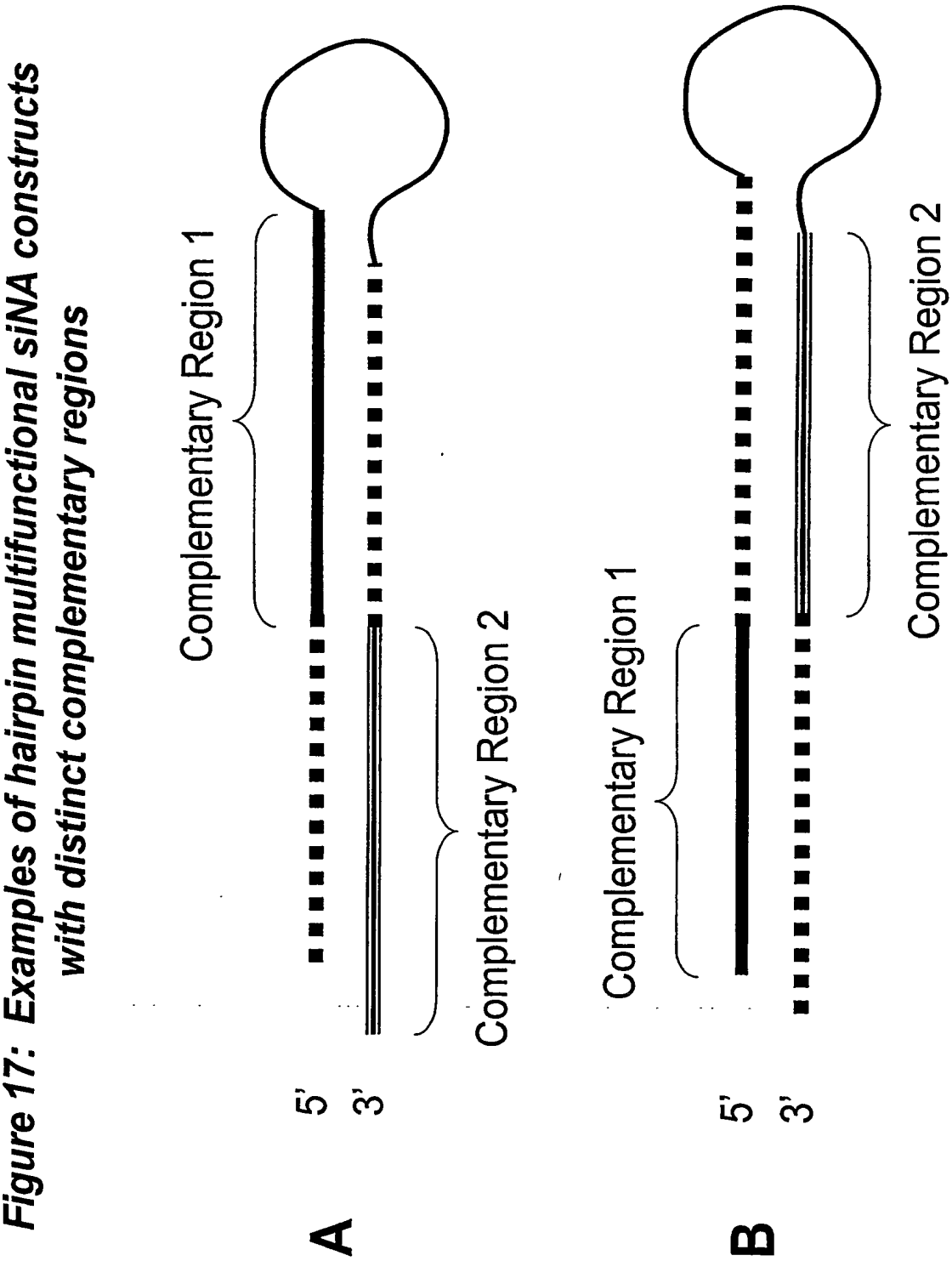
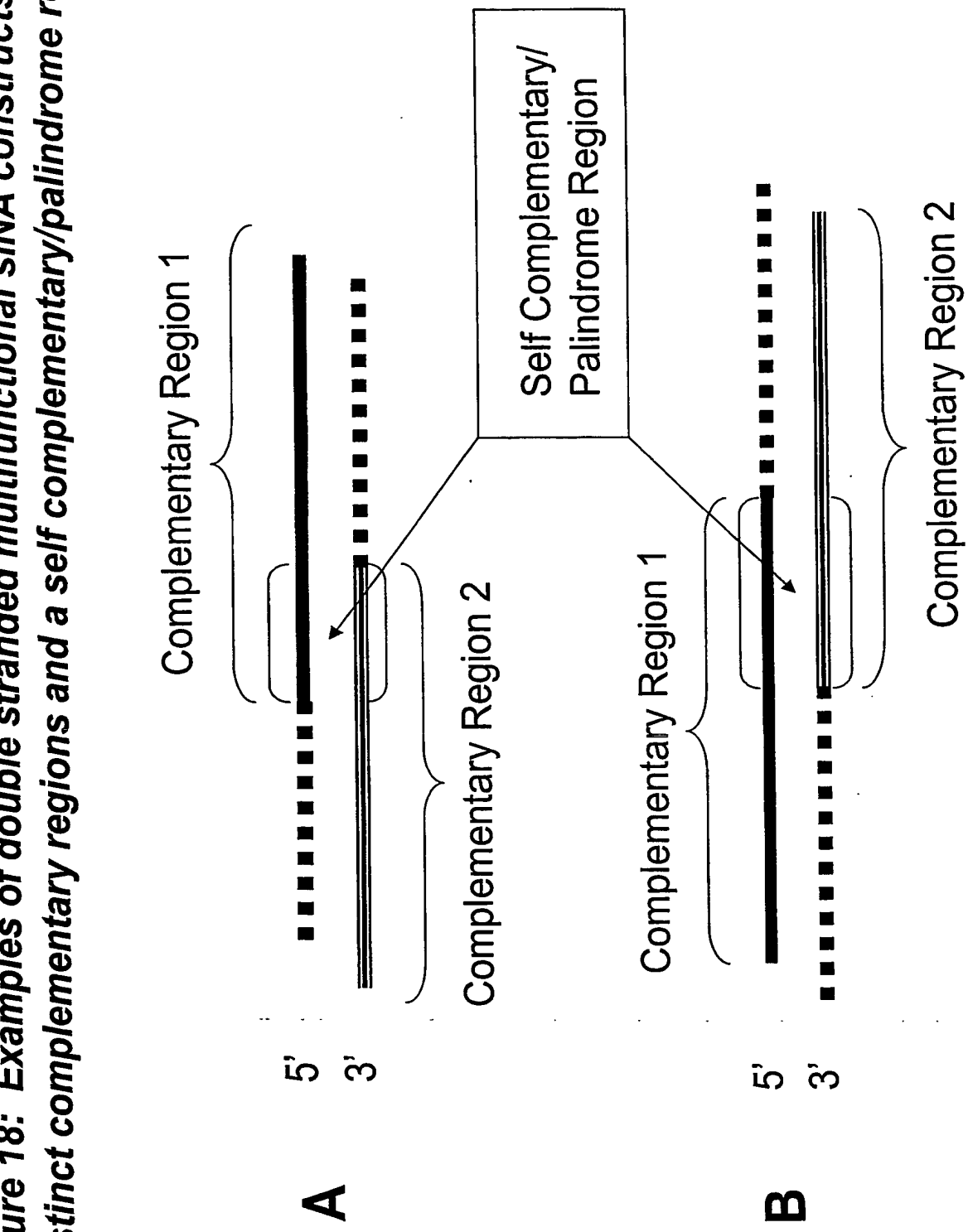


Figure 18: Examples of double stranded multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region



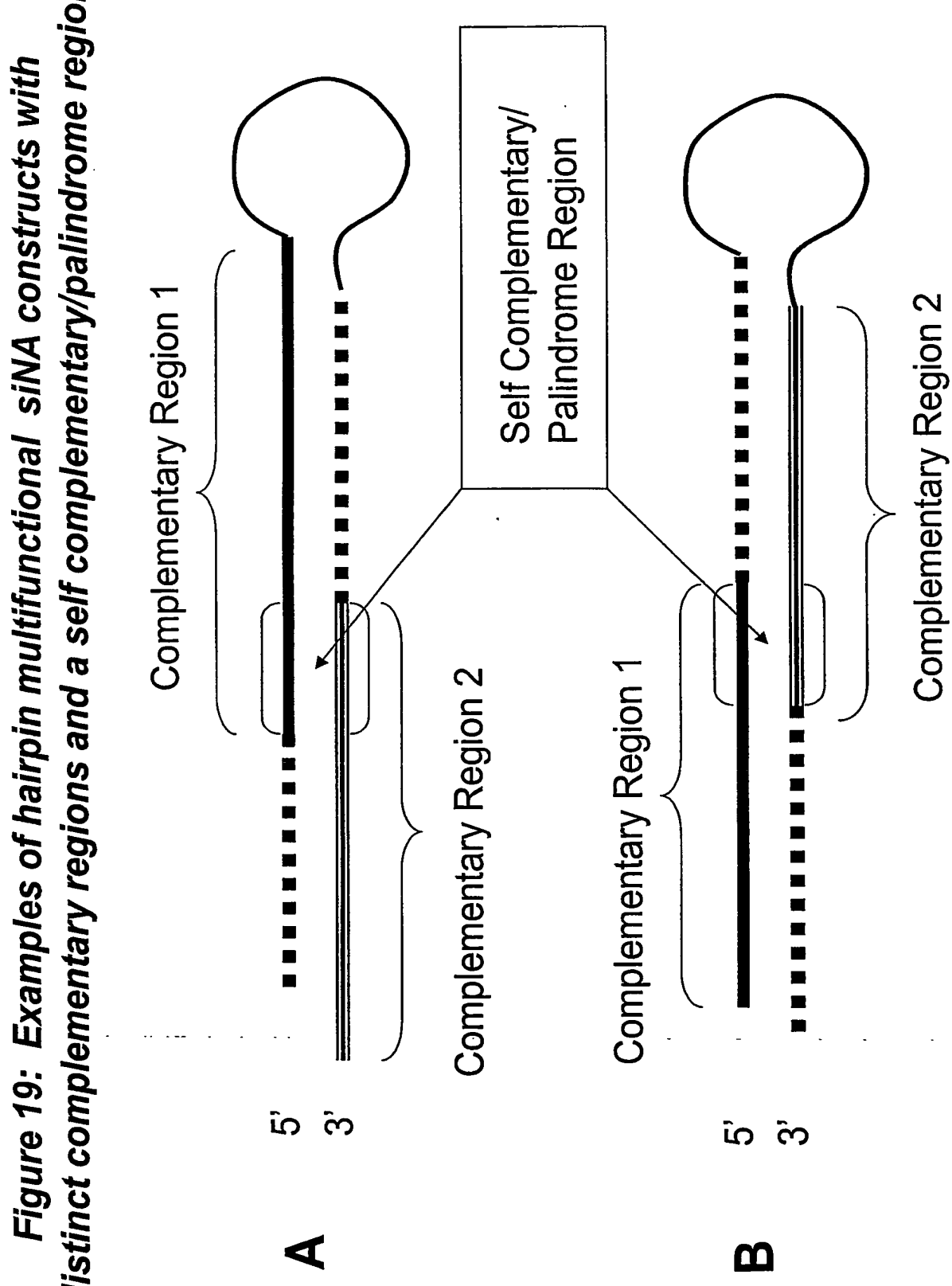


Figure 19: Examples of hairpin multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region

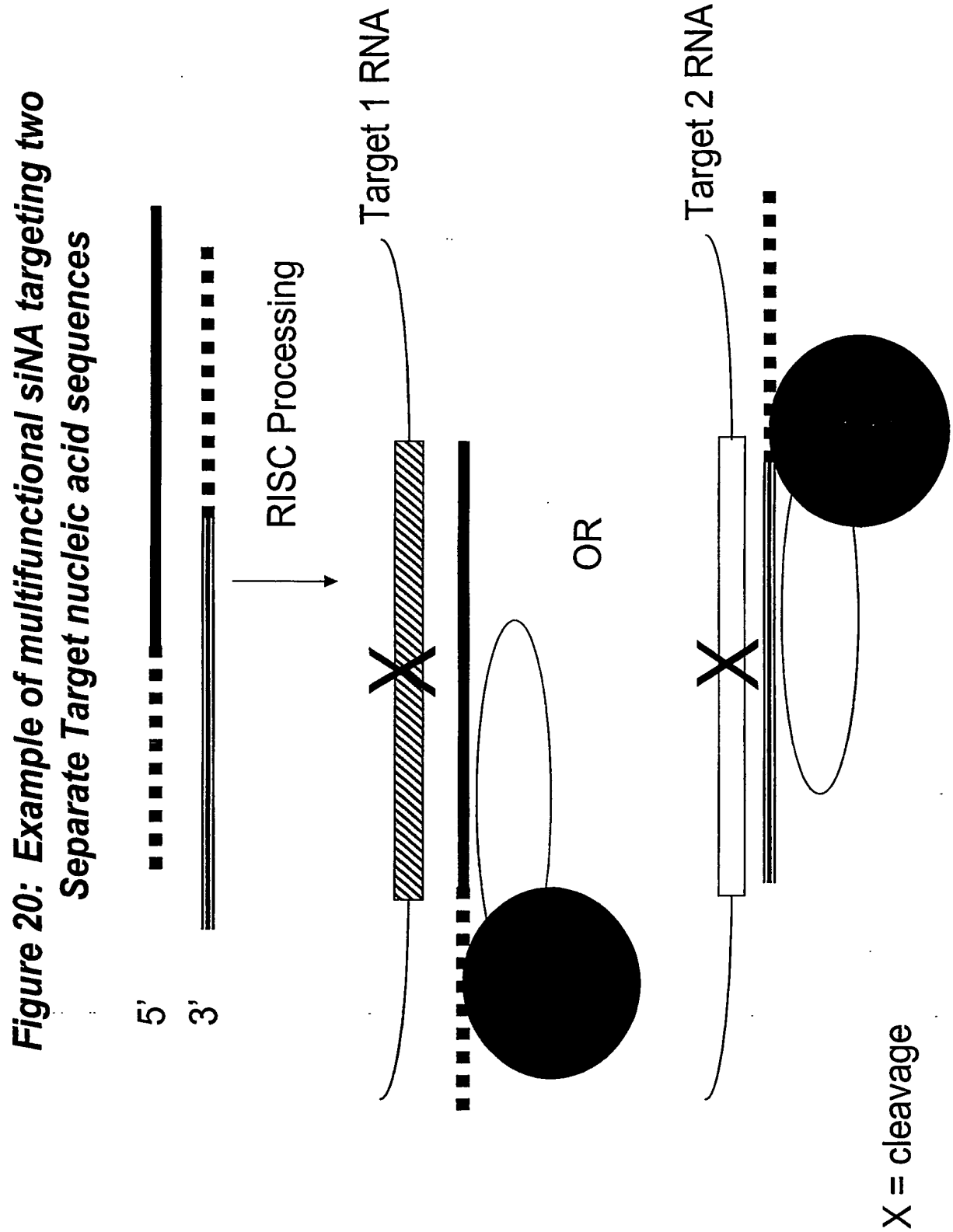


Figure 20: Example of multifunctional siNA targeting two Separate Target nucleic acid sequences

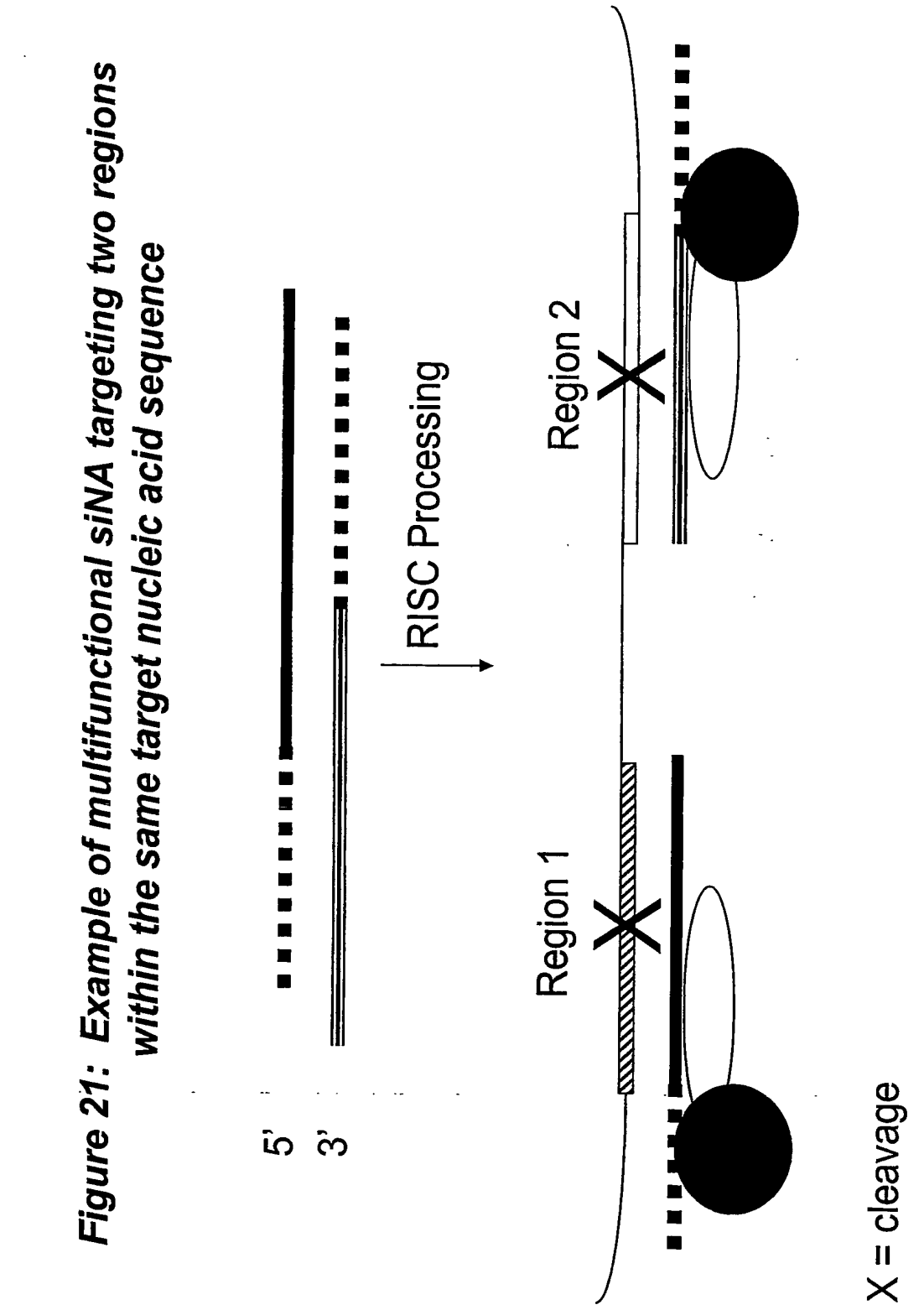
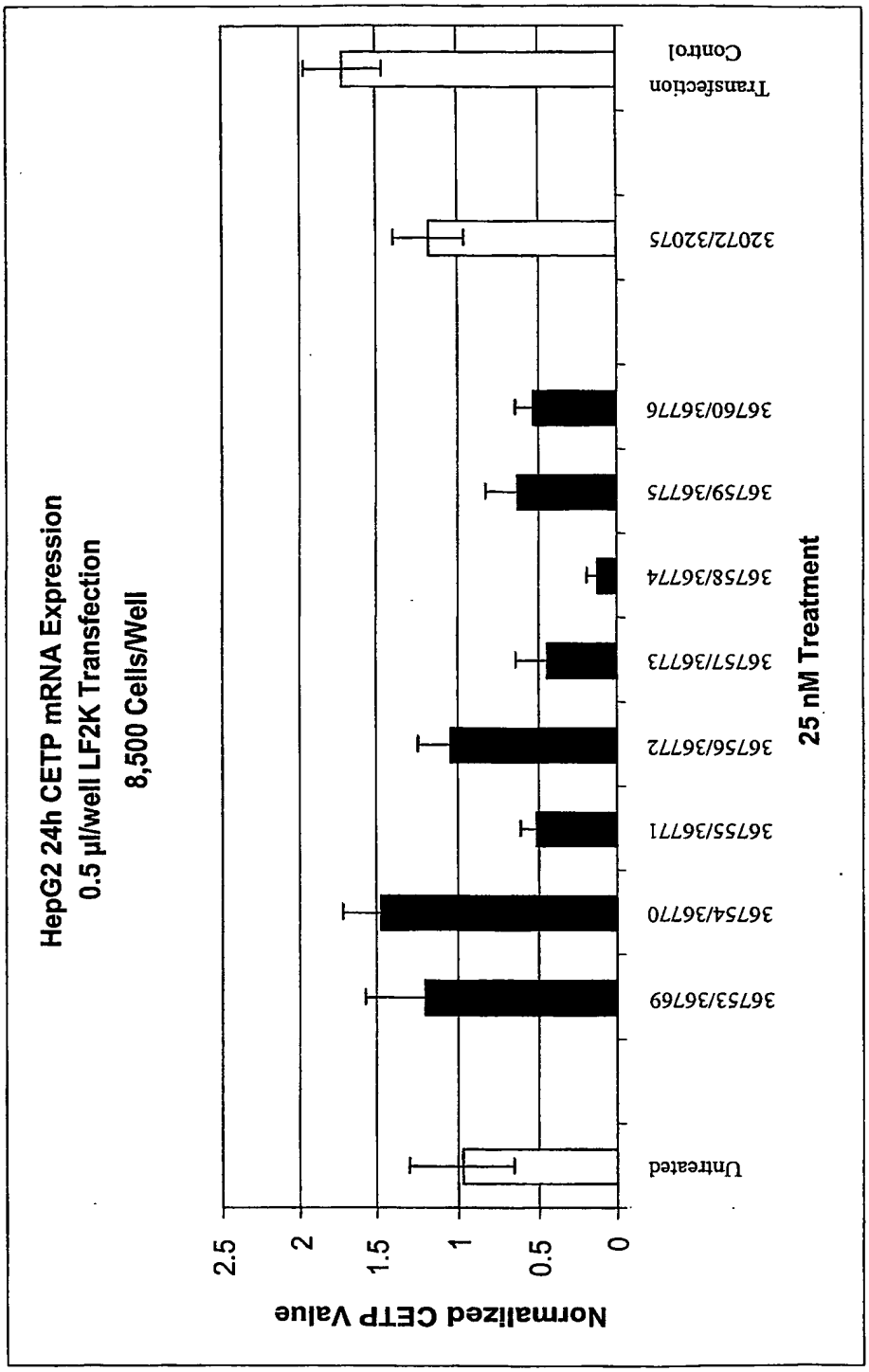


Figure 21: Example of multifunctional siNA targeting two regions within the same target nucleic acid sequence

FIGURE 22


HepG2 24h CETP mRNA Expression
0.5 µl/well LF2K Transfection
8,500 Cells/Well
Normalized CETP Value
2.5
2
1.5
1
0.5
0
Untreated
36753/36769
36754/36770
36755/36771
36756/36772
36757/36773
36758/36774
36759/36775
36760/36776
32072/32075
Transfection Control
25 nM Treatment